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Via Email

March 22, 2011

Danica Andrews
NIEHS
P.O. Box 12233, MD K2-03
Research Triangle Park, NC 27709

Re: Comments on Draft NTP Technical Report 575 on Acrylamide

Dear Ms. Andrews:

I am responding to your February 15, 2011 *Federal Register* notice (76FR 8741-8742) on "Availability of Draft NTP Technical Reports and Request for Comments" on acrylamide. I am an independent consultant in food, nutritional and chemical toxicology and safety and have been deeply involved with safety and regulatory issues surrounding heat-produced food toxicants since my graduate research studies begun in the mid-1970's. In addition I have been particularly involved with acrylamide's safety evaluation since its discovery in foods in April 2002.

After reviewing the Acrylamide Draft Technical Report (TR) 575, I wish to submit the following comments, sponsored by the Grocery Manufacturers Association (GMA) in Washington, DC. I understand that your NTP Technical Reports Peer Review Panel will meet on April 5 to discuss and evaluate several chemicals, including acrylamide. At this time I am not sure if I will be able to attend that meeting to present oral testimony, but I would appreciate if my comments could be made available to Peer Review Panel members prior to the meeting.

I. Draft NTP Technical Report on Glycidamide.

Before addressing specific comments and questions on acrylamide, I would like to ask why acrylamide is being peer reviewed without waiting for the release of the Draft NTP Technical Report on Glycidamide and why both chemicals are not being peer reviewed together. The U.S. Food and Drug Administration nominated both acrylamide and glycidamide, its principal metabolite, for NTP testing in November 2002. Because the toxicology of both chemicals has

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been recognized to be very closely associated for decades, and since NTP/NCTR researchers have expended very considerable resources studying the comparative metabolism and toxicokinetics of both chemicals, I believe it will be difficult to successfully evaluate and peer review the *Toxicology and Carcinogenesis Studies of Acrylamide* without simultaneously evaluating and peer reviewing the *Toxicology and Carcinogenesis Studies of Glycidamide*. Because the glycidamide database for the chronic study is not yet publicly available (i.e., the online pathology, survival and statistical tables) and appears to be lagging the acrylamide data release by almost a year (some preliminary acrylamide tables were available online in April 2010), I believe it would be inappropriate for the Peer Review Panel to make any final classification decisions on acrylamide's Levels of Evidence until their peer review and final decision making on the glycidamide chronic study are also completed.

II. Survival/Early Deaths – Was the Maximum Tolerated Dose (MTD) Exceeded?

Rats (see TR pages 67-71): It is reported that for the male rats, there was no effect of dose on survival and that the top dose body weights were 86% of control body weights. For the female rats, it is reported that there was a dose-related decreasing trend in survival at the top three doses and that the top dose body weights were 85% of control body weights. In explaining the primary causes of death in the rats (page 67), it is concluded that: "The primary cause (≥70%) for the early removal or death of these rats was neoplasms, including mononuclear cell leukemia, mammary gland fibroadenoma, clitoral gland adenoma or carcinoma, pituitary gland adenoma or carcinoma, and Zymbal's gland squamous cell carcinoma."

Mice (see TR pages 101-105): It is reported that for the male mice, the top dose had reduced survival and body weight decreases < 6%. For the female mice, it is reported that the top two doses had reduced survival and body weight decreases < 6%. In explaining the primary causes of death in the mice (page 101) it is concluded that: "The primary cause (>85%) for the early removal or death of these mice was neoplasms, including malignant lymphoma, leukemia (females only), mammary gland adenoacanthoma or adenocarcinoma (females only), harderian gland adenoma, and various types of sarcoma."

In making these conclusions that the primary causes of early removal or death in both rats and mice were the tumors cited above, several important questions come to mind:

- Was a rigorous cause-of-death analysis performed on these animals, or is the high attribution of percent deaths due to the cited tumors simply a best guess?
- How could this significantly decreased survival in rats and mice be caused mainly by benign tumors reported in the study?
- Was there a large enough number and severity of malignant tumors seen to have caused so many early deaths?
- Could the well-known central nervous system chronic toxicity of acrylamide have been a factor in some of the early deaths? Has acrylamide toxicity been ruled out?
- If some of this decreased survival was due to the high-dose, excess toxicity of acrylamide, was the Maximum Tolerated Dose (MTD) exceeded in this study?

While cause-of-death analysis may be onerous to accomplish, it is nonetheless an important aspect in trying to interpret certain bioassay studies. It is recognized that, even if the designation of tumors as either "fatal" or "incidental" is not completely accurate, the assessment may be a significant enhancement to the statistical analysis of tumor data (see, for example, Kodell et al., 1995).

Given these facts, I believe it is incumbent upon the NCTR researchers to describe their cause-of-death analyses in much greater detail and to provide their detailed justification of the high attribution of tumors as the cause of most of the deaths in the acrylamide bioassay.

III. Rat Mammary Gland Fibroadenomas – Use of Non-NCTR Historical Controls.

It is commonly accepted that historical control tumor data can be critical in the interpretation of rodent carcinogenicity studies, and particular care must be taken to ensure that historical controls are comparable to the concurrent control group with respect to those factors known to influence tumor occurrence. It is also well recognized that the most appropriate historical control data are those from relatively recent studies, if available, from the same study laboratory. Although the concurrent control group should be the primary control group used for bioassay decision making, there are instances in which historical control information from other NTP laboratories can also be useful in the interpretation of experimental results. Such historical control data may also be helpful in the determination of whether a marginal increase in tumor incidence relative to concurrent controls is a biologically meaningful effect. This is especially true when a specific tumor type, such as the rat mammary gland fibroadenoma, is recognized as a high-incidence spontaneous tumor occurring in untreated animals. In such instances, a statistically significant increase in tumors may be discounted if validated historical control data can demonstrate that the incidences seen in the dose groups are consistent with control values encountered in recent similar studies.

The Draft TR shows for rats and mice that NCTR has not done many recent drinking water bioassay studies in house, or if they have, they have chosen to use only older studies for historical control comparisons for acrylamide.

> **Rats**: only 7 bioassays are cited (TR pages 147-148 for males; pages 168-171 for females). These bioassays range from studies reported in 1988 to 2001, but no more recent studies are reported since then.
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> **Mice**: only 8 studies are cited (TR pages 187-188 for males; pages 205-208 for females). These bioassays range from studies reported out in 1988 to 2003, but no more recent studies are reported since then.

However, NTP has published online (in March 2010) very extensive and more recent Historical Control Data Tables for rat and mouse tumors for various routes of exposure. For the "Oral/Water" controls reported there for the NTP 2000 diet, NTP reported the female rat spontaneous mammary fibroadenoma incidence (mean $\pm$ standard deviation) to be 74% $\pm$ 12%. The concurrent controls in the acrylamide study were reported to be 33% (Table 15, page 80), while the NCTR historical controls (Table B3c, page 169) were reported to be 35.0% (range

27.1% - 42.6%). A comparison of these control data to the increasing dose incidences reported for acrylamide treatment is given here:

	Rat Mammary Gland Fibroadenoma % Incidence
Acrylamide concurrent controls:	33%
NCTR historical controls:	35% (27.1 - 42.6%)
Recent NTP Oral/Water controls:	74% ± 12%
Acrylamide doses (lowest to highest):	38% 52% 47% 65%

While it is apparent from this comparison that the highest acrylamide dose (65% incidence) was significantly higher than both the concurrent controls and the NCTR historical controls, the incidence rates of the rat mammary gland fibroadenomas for all four acrylamide doses fall well below the incidences reported for the much more contemporary NTP Oral/Water historical controls.

Therefore, I believe that the Peer Review Panel should look more closely into these more contemporary historical controls for NTP drinking water bioassays as a more appropriate historical control database for evaluating whether acrylamide treatment in this study is a plausible biological explanation for these female rat benign mammary tumors.

Thank you for this opportunity to submit these comments on the Draft NTP Technical Report on Acrylamide.

Sincerely,

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